Original Article

Evaluation of antimicrobial efficacy of *Aloe vera* and *Meswak* containing dentifrices with fluoridated dentifrice: An *in vivo* study

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Abstract

Aim: To comparatively evaluate the antimicrobial efficacy of fluoridated and herbal dentifrices. **Materials and Methods:** Sixty students in the age group 6–12 years with DMF/def score 0 were selected from an orphanage center. The participants were divided into four groups. In group A, no dentifrice was used; in group B, fluoride containing dentifrice was used; group C subjects used *Aloe vera* containing dentifrice; and in group D, *Meswak* containing dentifrice was used. The salivary samples were collected at the washout period of 2 days, 15 days, and 30 days and cultured on Mitis Salivarius Agar for determining *Streptococcus mutans* count. Results obtained were statistically analyzed using Student's *t*-test. **Results:** There was an increase in bacterial count in group A where no dentifrices were used, while the bacterial count steadily decreased in groups B, C, and D by 83.7%, 80.94%, and 83.5%, respectively. **Conclusion:** Herbal dentifrices containing *A. vera* and *Meswak* can be safely recommended as an alternative to fluoridated dentifrices in terms of antimicrobial efficacy.

Key words: *Aloe vera*, *fluoride*, *Meswak*

INTRODUCTION

Dental caries is a common chronic disease, arising from interplay between oral flora, teeth, and diet. According to Keyes triad (1960),[1] which later on was modified by Newbrun (1982) into tetrology, the interaction between these three primary factors over a specified time period is essential for the initiation and progression of caries.[1] Hence, if any one of the factors is eliminated or reduced, the prevalence of dental caries can be reduced.

Microorganisms play a vital role in the causation of dental caries. Those which are capable of converting sucrose to lactic acid play a lead role, such as *Streptococcus mutans*, *Lactobacillus*, etc., *S. mutans*, colonizing the oral cavity, is considered to be associated with initiation of dental caries.

Fitzgerald, Jordan, and Achard (1964)[2] demonstrated that dental caries will not occur in the absence of microorganisms. Complete removal of microorganisms from the oral cavity is impossible, but reduction in microbial count may reduce the cariogenic effect. Oral cavity is an ecological niche, which contains 500–1000 different types of bacteria along with fungi, protozoa, and occasionally viruses. Oral microflora increases due to frequent intake of sucrose

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and carbohydrate and poor oral hygiene and decreases due to various preventive measures such as topical fluoride application, pit and fissure sealants, etc., The mechanical measures used in our daily routine, such as tooth brushing, dental flossing, etc., if complimented with a dentifrice having antimicrobial efficacy, can reduce the microbial count of the oral cavity on a daily basis and, thus, reduce the prevalence of dental caries.

Acharya *et al*.[3] assessed the antimicrobial activity of different toothpastes against oral isolates by conducting zone of inhibition test and concluded that antimicrobial agent is present in various test dentifrices.

Dentifrices containing fluoride are commercially available since 1980s. Fluoride usage and decline in the prevalence of dental caries in developed countries is mainly attributed to its increased use. Torell, Ericsson (1965),[1] Koch (1967),[1] Lind *et al*. (1974),[1] and various other studies[4,5] concluded that fluoride acts as a major antimicrobial ingredient. But use of these dentifrices in high fluoridated belt can cause fluoride toxicity. Hence, to avoid the toxic effect of fluoride in case of overdosage and for its restricted usage in children, alternative dentifrices were searched for.

Herbs have been used in India and South Asia for thousands of years to clean and fight bacterial and fungal infections. Modern science validates that *Aloe vera* and *Meswak* have antimicrobial properties. Studies suggest that *A. vera* and *Meswak* extract is appropriate for treating gingivitis and oral infections, as it inhibits the formation of plaque and the growth of bacteria. Oliveria *et al*.[6] conducted a double-blind clinical study in humans on the effect of dentifrice containing *A. vera* on plaque and gingivitis control, in which fluoridated dentifrices were taken as controls; both control and test dentifrices showed significant reduction in plaque and gingival index over a period of 1 month.

The present study was conducted on commercially available dentifrices, namely a non-herbal fluoridated dentifrice – Colgate – and two herbal dentifrices – Forever Bright Aloevera Toothgel and Dabur Meswak toothpaste – with the objective of evaluating the antimicrobial activity of the dentifrices used and quantitatively correlating it with oral microflora.

MATERIALS AND METHODS

Study design

An ethical clearance from the ethical committee (DJD/IEC/9/9/YEAR/2011) was taken prior to the commencement of the study. The study was conducted in Grace home center, an orphanage, in Ghaziabad district. A total of 60 children of age group of 6–12 years who fulfilled the inclusion and the exclusion criteria were selected to participate in the study.

Inclusion and exclusion criteria

Inclusion criteria included children who had DMF/def score zero.

The exclusion criteria for selection included presence of any marked intraoral soft tissue pathology, subjects with history of taking antibiotics 3 months prior to or during the course of study, medically compromised patients, children undergoing orthodontic therapy, and children with history of professionally applied topical fluoride.

Division of samples

Consent was taken from the orphanage authority for conducting the study on the participants selected and an agreement was made not to use any other oral hygiene products than those assigned during the study, including mouthrinses, dentifrices, whitening or therapeutic chewing gums, whitening formulations, etc., Participants were instructed not to visit any dental surgeon during the study period. Not participating in other studies was agreed upon by the participants.

The study subjects were provided with same toothbrushes (oral B kid) and were demonstrated same tooth brushing technique (Fones' technique). The time (2 min), duration (twice daily), and amount of dentifrice were also kept same for all children to maintain standardization, which was personally monitored.

Participants were divided into groups by utilizing simple random sampling method. In this, numbers were allotted to all children selected for the study and using a random number table,[7] the selected children were divided into four groups, each consisting of 15 subjects as follows: Group A: Without using dentifrices, group B: Fluoridated dentifrice Colgate, group C: Herbal dentifrice Forever Bright Aloevera Toothgel, and group D: Herbal dentifrice Dabur Meswak toothpaste. The study took place over a period of 1 month. Saliva collection (to assess the microbial count) was performed at baseline and after a washout period of 2 days, 15 days, and 30 days. A washout period of 2 days was given after the baseline count, wherein the children brushed their teeth with their regular brush, but without a dentifrice to nullify the effect of previous dentifrices used.

Saliva collection method

Stimulated saliva samples were obtained from the subjects by spitting method, as the stimulated saliva samples yielded significantly higher levels of *St. mutans* (about 1.5 log10 increase) with a lower variability, compared to unstimulated saliva samples.[8]

Three students at a time were made to sit comfortably on a chair. After swallowing pre-existing saliva, the subjects were given paraffin wax to chew to stimulate salivary flow, which was then collected by expectorating in a sterile disposable measuring cup over the next 5 min.

Standardization of the saliva collection technique was done by asking the subjects not to perform any physical exercise and not allowing them to eat or drink (except water) 1–2 h before salivary sample collection. The saliva was collected over a period of 5 min and paraffin block was used as the stimulant.

Samples were transported for microbiological evaluation in dry ice (at −70°C) within 6–8 h to Max Super Speciality Hospital, Saket.

St. mutans count was done by culturing the salivary sample in selective culture media (Mitis Salivarius Agar).

Laboratory procedures

The samples were vortexed to uniformly mix the saliva. Using an inoculation loop (standard loop with 4 mm diameter), 10 µl of the vortexed sample was streaked on Mitis Salivarius Bacitracin agar selective for *St. mutans*. The Mitis Salivarius Agar plates were incubated in aerobic conditions at 37°C for 48 h in an incubator. Gram staining and catalase test were conducted on colonies with morphologic characteristics of *St. mutans* (0.5 mm raised convex undulated colonies of light blue color with rough margins, granular frosted glass appearance) [Figure 1], and the colonies were finally identified by using vitek 2 compact bacteria identifying machine. These *St. mutans* colonies on the plate were counted using a colony counter machine and were expressed as number of colony forming units per milliliter (CFU/ml) of saliva. Semi-quantitation of the number of colonies was done by multiplying the actual colony count with 1×10^3, as the sample was diluted 1 in 10 times (1:10 dilution).

Statistical analysis

The data obtained were statistically analyzed using SPSS software, version 16. Student's *t*-test was applied at 95% confidence level ($P \leq 0.05$, significant).

Percentage values were taken, as the baseline values of the sample were not uniform.

RESULTS

In all the groups, the mean percentage (%) value of CFU/ml increased after the washout period of 2 days, i.e., in group A (without using dentifrice), group B (fluoridated dentifrice Colgate), group C (herbal dentifrice Forever Bright Aloevera Toothgel), and group D (herbal dentifrice Dabur Meswak Toothpaste), it increased by 79.10%, 79.6%, 82%, and 80%, respectively [Table 1 and Graph 1].

After the washout period of 2 days, a continuous reduction in microbial count was observed in groups B, C, and D where dentifrices had been used, at various intervals, i.e. 15 days and 30 days. In total, the mean percentage of CFU/ml count of groups B, C, and D had shown reduction by 83.7%, 80.95%, and 83.5%, respectively. In group A, where no dentifrice was used, a constant increase in CFU/ml count was seen, i.e. it increased by 55.93% after the washout period [Table 1 and Graph 1].

The *P* value was found to be nonsignificant when intercomparison between groups B, C, D was done applying Student's *t*-test [Table 2].

DISCUSSION

Microorganisms play a vital role in causation of dental caries. Various studies[2] have provided evidence of bacterial specificity in caries etiology. Complete removal of microorganisms from the oral cavity is impossible, but their count can be reduced so that it becomes less cariogenic with the help of various preventive measures,

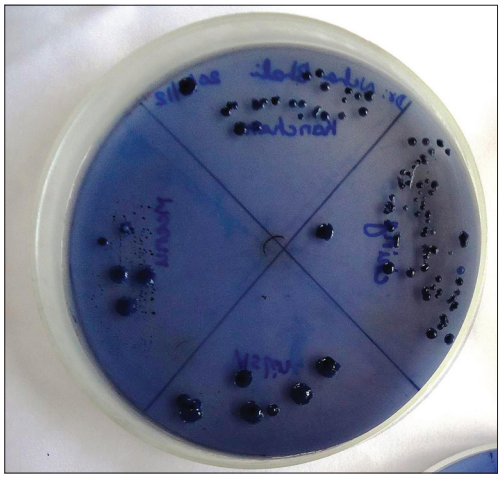


Figure 1: Colony morphology of *S. mutans* on Mitis Salivarius Agar

Table 1: Mean percentage increase or decrease of colony forming units per milliliter (CFU/ml) of *S. mutans* in groups A, B, C, and D

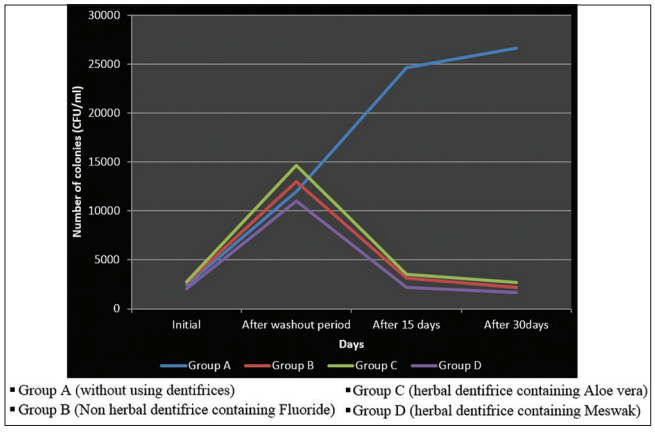
Mean % of *S. mutans* reduction	Mean % value ± SD			
	Group A	Group B	Group C	Group D
% increment b/w initial value and washout period	+79.10±5.053564916	+79.6511±8.14493	+82.0038±5.41107	+80.3968±5.05184
% reduction/increase b/w washout period and 15th day	+52.4154±8.43560	−76.2923±7.86728	−75.0105±7.20684	−78.1581±8.66329
% reduction/increase b/w 15th day and 30th day	+7.90±7.661773056	−27.0159±19.53145	−23.1111±16.24303	−23.8889±29.18650
Total % of reduction/increase from washout period and till 30th day of using paste	+55.93±9.614031729	−83.7119±4.08042	−80.9489±5.79849	−83.4999±9.01218

Reduction = −, Increase = +, Group A=Without using dentifrices; group B=Fluoride containing dentifrice; group C=Aloe vera containing dentifrice; group D=Meswak containing dentifrice. CFU/ml=Colony forming units per milliliter

Table 2: Intercomparison of mean percentage increase or decrease of colony forming unit count (CFU/ml) of *S. mutans* in groups B, C, and D after 30 days

Comparisin of mean percentage bacterial count change	Groups	*n*	Mean	Std. deviation	Std. error mean	*P* value
% reduction b/w washout period and 30 days	B	15	−83.7119	4.08042	1.05356	0.142
	C	15	−80.9489	5.79849	1.49716	
% reduction b/w washout period and 30 days	B	15	−83.7119	4.08042	1.05356	0.934
	D	15	−83.4999	9.01218	2.32694	
% reduction b/w washout period and 30 days	C	15	−80.9489	5.79849	1.49716	0.364
	D	15	−83.4999	9.01218	2.32694	

P≤0.05 – Significant. P≥0.05 – Non-significant. Group B=Fluoride containing dentifrice; group C=Aloe vera containing dentifrice; group D=Meswak containing dentifrice. CFU/ml=Colony forming units per milliliter



Graph 1: Line graph representing increase and decrease of colony forming units per milliliter (CFU/ml) of *S. mutans* at various intervals

for example, probiotics, antibiotics, fluorides, and oral hygiene aids.

Various oral hygiene measures are available, such as tooth brushing, dental flossing, mouthwashes, dentifrices, etc., among which tooth brushing with dentifrice is the most commonly used. These mechanical measures are feasible, cost-effective, and can easily be used by children. Dentifrices are therapeutic mechanical aids which are available as tooth powder or toothpaste and aid in removal of plaque. Their antimicrobial effect has been proven in various studies. Leyster *et al*.[9] conducted an *in vitro* study to investigate the antimicrobial efficacy of commercial dentifrices on *St. mutans* and *Lactobacillus* and concluded that an active antimicrobial ingredient was present in oral dentifrices.

In fluoridated dentifrice, fluoride is mostly available in the form of sodium fluoride or sodium monofluorophosphate. Fluoride acts by inhibiting cellular enzymes (direct binding of F^- or hydrogen fluoride, or in combination with metals) or enhances the proton permeability of cell membranes in the form of hydrogen fluoride (acting as a transmembrane proton carrier). Hydrogen fluoride enters the bacterial cell membrane and dissociates to yield hydrogen and F^-. Intracellular F^- inhibits glycolytic enzymes, resulting in decreased acid production from glycolysis. Also, it lowers cytoplasmic pH, affecting the acid production and acid tolerance of mutans streptococci. But use of these dentifrices causes abrasion of teeth as along with fluorides, they also contain abrasive agents. Moreover, these pastes are not recommended in high fluoridated belt and also in children under 3 years of age. Thus, the quest for a dentifrice which has less abrasive effect, less chemical agents, and more antimicrobial property made the researchers focus on age-old medicinal alternative "herbs."

Various herbal ingredients in oral health care products had been used as cariostatic agents, analgesics,

antimicrobials, or bleaching agents. Among the various currently available herbal agents, *A. vera*, popularly known as "*Babosa*," is a plant commonly found the northeast of Brazil.[6] Its foliage, extract, and resin exhibit antimicrobial, anti-inflammatory, and healing properties and are indicated for hepatic and stomach diseases as well.[6] The antimicrobial effect of a dentifrice containing *A. vera* has been demonstrated in an *in vitro* study, in which this phytotherapeutic agent inhibited the growth of diverse oral microorganisms such as *St. mutans*, *Streptococcus sanguis*, *Actinomyces viscosus*, and *Candida albicans*.[6] *A. vera* contains specific plant compounds such as anthraquinones and dihydroxyanthraquinones as well as saponins which have been proposed to have direct antimicrobial activity and act by inhibiting protein synthesis by bacterial cells.[6]

Meswak herb is a rare, potent, priceless wonder herb that delivers incredible oral care benefits. It is scientifically proven to reduce tartar and plaque, fights germs and bacteria to keep the gum healthy, helps prevent tooth decay, eliminates bad breath, and ensures strong teeth.[10] Meswak is a herbal dentifrice containing the pure extract of the *Meswak* plant Salvadora persica, the famous "Toothbrush Tree" which has been used for centuries. The astringent and antibacterial properties of Meswak help reduce tooth decay, fight plaque, and prevent gum diseases. Moreover, these products do not have any abrasive agents. In *Sa. persica (Meswak),* a natural component benzylisothiocyanate (BIT) is present that acts as an inhibitor of bacterial growth and their acidic products. Also, the antibiotic effects found in *Meswak* may prevent the attachment of bacteria.[10]

In the present study, group A (without using dentifrices) showed an increase in bacterial count (CFU/ml) when evaluated from the washout period of 2 days till the cessation of the study (i.e. 30 days) and the mean percentage increase was 55.93%. Thus, it reinforces the antimicrobial effect of dentifrices. The studies of Binney *et al*. (1993), Parizotto *et al*. (2003), and Paraskevas *et al*. (2006) showed that use of dentifrices did not contribute to additional plaque removal during manual tooth brushing and that mechanical action of tooth brushing is sufficient in maintaining oral hygiene.[11] But in the present study, the age group taken was 6–12 years, wherein dexterity is not as adequate as those of adults. This might be the reason behind the constant increase in antimicrobial count in the group where no dentifrices had been used.

Significant reduction in the bacterial colony count was seen in group B (fluoridated dentifrice Colgate), group C (herbal dentifrice Forever Bright Aloevera Toothgel), and group D (herbal dentifrice Dabur Meswak Toothpaste), which was 83.7%, 80.95%, and 83.5%, respectively, from the washout period till the cessation of the study (i.e., at 30 days). This is similar to the results obtained in a previous study by Patil *et al*.[12] in which they did an *in vivo* comparison of two commercially available dentifrices (cheerio gel and Himalaya herbal dental cream containing neem) on the salivary *St. mutans* count in urban preschool children and found a steady reduction of bacterial count with both fluoridated dentifrices as well as herbal (neem-containing) dentifrice.

When intercomparison of mean *St. mutans* count was done between groups where dentifrices were used, the result was found to be nonsignificant ($P > 0.05$) at the interval of 30 days of the study [Table 2]. This indicates that all the three test dentifrices (tooth brushing using non-herbal fluoridated dentifrice, *A. vera* containing dentifrice, and *Meswak* containing dentifrice) have equal antimicrobial efficacy.

Similar results were obtained in the study conducted by George *et al.* (2009),[13] Almas K (2001[14] and Patil).[12]

In the present study, no significant difference was seen in the antimicrobial property of all the three test dentifrices. Hence, we can safely recommend *A. vera* and *Meswak* containing dentifrice as an alternative to fluoridated dentifrice for children.

Although the bacterial count had shown reduction in 15 days and remained the same over a period of 1 month (30 days), the prolonged effect on the bacterial count, as well as whether the reduced count is stabilized or the counts return back to baseline values need to be checked. Whether there is any bacterial resistance developing to these products also needs to be monitored carefully in further studies.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Newbrun E. Cariology. 3rd ed., Ch. 2. Chicago: Quintessence Publishing Co Inc.; 1989. p. 29, 37.
2. McDonald RE, Avery DR, Dean JA. Dentistry for the Child and Adolescent. 8th ed. Ch 10: Mosby; 2004. p. 205.
3. Acharya A, Subedi B, Paudyal B, Jnawali M, Shakya P, Shrestha UT, *et al.* Assessment of antimicrobial activity of different dentifrices against oral isolates. Planta Med 2006;68:1-11.
4. Auschill TM, Deimling D, Hellwig E, Arweiler NB. Antibacterial effect of two toothpastes following a single brushing. Oral Health Prev Dent 2007;5:25-32.
5. Sensabaugh C, Sagel ME. Stannous fluoride dentifrice with sodium hexametaphosphate: Review of laboratory, clinical and practice-based data. J Dent Hyg 2009;83:70-8.
6. De Oliveira SM, Torres TC, Pereira SL, Mota OM, Carlos MX. Effect of a dentifrice containing Aloe vera on plaque and gingivitis control. A double-blind clinical study in humans. J Appl Oral Sci 2008;16:293-6.
7. Hill AB. A Short Textbook of Medical Statistics. 10th ed., Ch. 6. Philadelphia: Lippincott Company; 1977. p. 103
8. Dasanayake AP, Caufield PW, Cutter GR, Roseman JM, Köhler B. Differences in the detection and enumeration of mutans streptococci due to differences in methods. Arch Oral Biol 1995;40:345-51.
9. Leyster CW. An investigation of the levels of antimicrobial efficacy in commercial dentifrices on *Streptococcus Mutans* and *Lactobacillus*. St Martin's Univ Bio J 2006;1:155-66.
10. Ahmad H, Ahamed N. Therapeutic properties of Meswak chewing sticks: A review. Afr J Biotechnol 2012;11:14850-7.
11. Jayakumar A, Padmini H, Haritha A, Reddy KP. Role of dentifrice in plaque removal: A clinical trial. Indian J Dent Res 2010;21:213-7.
12. Patil S, Venkataraghavan K, Anantharaj A, Patil S. Comparison of two commercially available dentifrices on the salivary Streptococcus Mutans count in urban preschool children-An *in vivo* study. Int Dent 2010;12:72-82.
13. George D, Bhat SS, Antony B. Comparative evaluation of the antimicrobial efficacy of Aloe vera tooth gel and two popular commercial toothpastes: An *in vitro* study. Gen Dent 2009;57:238-41.
14. Almas K. The antimicrobial effects of seven different types of Asian chewing sticks. Odontostomatol Trop 2001;24:17-20.